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Improvement of a Robust HPLC Method for Abemaciclib and Its Process-Related Pollutants; Degradation Product Identification Using LCMS/MS

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Abstract: *The current study was undertaken to explore an innovative approach that employs liquid chromatography (LC) and liquid chromatography-mass spectrometry (LCMS/MS) for the resolution, identification, and characterization of minute quantities of degradation products (DPs) of abemaciclib, without the need for their isolation from reaction mixtures. This method successfully separated process-related impurities, including abemaciclib, on Zorbax XDB (250×4.6 mm; 5 µ id) C18 column at room temperature using 0.2 M phosphate buffer with pH4.2 and methanolin 80:20(v/v)asmobilephaseA,0.2M phosphate buffer with pH 4.2 and acetonitrile in the ratio of 30:70 (v/v) as mobile phase B. The mobile phase solvent A andBweremixedat50:50(v/v) and the mixture was pumped is ocratically at1.0mL/min and UV detection at 238 nm. The method demonstrates a sensitive detection limit of 0.09 µg/mL for the studied impurities, with a linear calibration curve spanning the range of 30 – 210 µg/mL for abemaciclib and 0.3 – 0.21 µg/mL for impurities. In accordance with the International Conference on Harmonization (ICH) guidelines, stress studies were conducted on the pure abemaciclib compound. Under various stress conditions, the drug exhibited instability in acidic, alkaline, and UV light environments, while maintaining stability in thermal and peroxide conditions. Six degradation products (DPs) were identified, with their fragmentation patterns and masses elucidated through LCMS/MS. The formed DPs were characterizedand confirmed as 5-(piperazin-1-ylmethyl)pyridin-2-amine (DP 1), 5-fluoro-4- [4-fluoro-2-methyl-1-(propan-2-yl)-1H-benzimidazol-6-yl]pyrimidin-2-amine(DP2),4-(4-amino-3-fluorophenyl)-N-[5-(aminomethyl)pyridin-2-yl]-5-fluoropyrimidin-2-amine(DP3), N-[5-(aminomethyl)pyridin-2-yl]-5-fluoro-4-(4-fluoro-2-methyl-1H-benzimidazol-6- yl)pyrimidin-2-amine(DP4),5-(2-amino-5-fluoropyrimidin-4-yl)-3-fluoro-N1-(propan-2- yl)benzene-1,2-diamine (DP 5), and 5-fluoro-4-(4-fluoro-2-methyl-1H-benzimidazol-6-yl)pyrimidin-2-amine (DP 6). The suggested approach proved effective for the routine analysis of abemaciclib and its process-related impurities in both pure drug samples and formulations. Additionally, it demonstrated utility in identifying both known and unknown impurities associated with abemaciclib.*

Keywords: abemaciclib, process related impurities, HPLC analysis, forced degradation studies, degradation products, LCMS/MS characterization

1. Introduction

Abemaciclib is a pharmaceutical compound that belongs to a class of drugs known as cyclin- depend entkinase(CDK)inhibitors [1]. Itisusedprimarilyin the treatment of certain types of cancer, with a focus on breast cancer. Abemaciclib works by inhibiting specific enzymes called CDKs, particularly CDK4 and CDK6. These enzymes play a crucial role in cell cycle regulation [2]. By inhibiting CDKs, abemaciclib helps slow down or halt the

uncontrolled growth of cancer cells. Common side effects of abemaciclib may include diarrhea, fatigue, nausea, abdominal pain, and decreased appetite. Diarrhea is a notable side effect and is often managed with dose adjustments or anti-diarrheal medications [3]

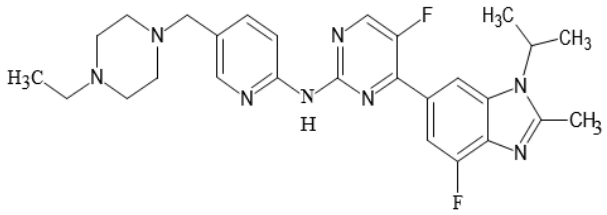


Figure1: Molecular structure of abemaciclib

HPLC impurity analysis plays a pivotal role in pharmaceutical, chemical, and various other industries due to its significant impact on product quality, safety, and regulatory compliance. Impurity analysis was treated as crucial in ensuring the quality and purity of pharmaceuticals and chemicals. It helps identify and quantify impurities, ensuring that products meet established specifications and regulatory standards. This is especially criticalin pharmaceuticals, where even trace impurities can impact a drug's efficacy and safety. The regulatory agencies such as the FDA and EMA mandate stringent impurity analysis as a part of drug approval and post-marketing surveillance. Compliance with these regulations is essential for market approval and ongoing product safety [4]. The impurity profiling helps detect potentially harmful or toxic substances in products. Identifying and controlling impurities ensures the safety of consumers and the efficacy of the product, especially in the case of pharmaceuticals, where impurities can have adverse effects on patients. Impurity analysis is vital in stability studies, helping manufacturers determine a product's shelflife and storage conditions. It ensures that a product remains safe and effective throughout its intended lifespan [5]

2. Materials and Methods

Reagents and solvents:

The analytical standard compound abemaciclib with purity of 98.52%, its impurity1, 2 and 3 were procured from Eli Lilly & Co Pvt. Ltd., Gurgaon, Haryana. The tablet formulation containing 100 mg of abemaciclib with brand VERZENIO® was purchased from local market. HPLC-grade methanol, acetonitrile, and Milli-Q® water were procured from Merck Chemicals, Mumbai. Analytical reagent-grade chemicals, including acetic acid, sodium acetate, hydrochloric acid (HCl), sodium hydroxide (NaOH), and hydrogen peroxide, were sourced from Fisher Scientific, Mumbai.

Instrumentation

HPLC study was conducted using an Agilent (USA) 1100 instrument equipped with a quaternary pump (G1311 A) for solvent delivery. Analytes were introduced through a temperature-adjustable autosampler(G1329A) with an injection capacity ranging from 0.1 to 1500 µL. Column eluents were detected using a programmable ultraviolet (UV) detector (G 1314 A), and chromatographic data were integrated using Agilent Chem Station software. LCMS analysis was carried out on a Waters LCMS system (Japan) featuring a triple quadrupole mass detector and MassLynx software

Standard solution preparation

Standard solutions of abemaciclib and its impurities were prepared at a concentration of 1 mg/mL (1000 µg/mL) individually. This was accomplished bypreciselyweighing25 mg of each analyte into separate 25 mL volumetric flasks, each containing 15 mL of methanol. The analytes were then dissolved in the solvent using an ultrasonic bath sonicator. Subsequently, the solutions were filtered through a 0.2 µm membrane filter, and the final volume was adjusted to the mark using the same solvent, resulting in a concentration of 1000 µg/mL for both abemaciclib and its impurities. During the analysis, the required volume of each analyte's specific concentration was used separately.

Testsolution preparation:

VERZENIO® tablets with strength of 100 mg were employed in the formulation solution preparation. The tablets were finely powdered using a clean and dry mortar and pestle, and an accurate amount of tablet powder equivalent to 25 mg of abemaciclib was weighed. The measured tablet powder was placed in a 25 mL volumetric flask containing 15 mL of methanol. Subsequently, the drug was completely dissolved in the solvent, and thefinal volume was adjusted to the mark. The solution was then filtered, diluted to a standard concentration, and the resulting dilute solution was utilized for the assessment of formulation assay.

3. Method development

The optimal detector wavelength for the maximum detection of both abemaciclib and its impurities was determined using a UV-visible spectrophotometer. A standard solution containing abemaciclib and its impurities at a concentration of 10 µg/mL was individually scanned using the spectrophotometer, and the resulting absorption spectra were examined to identify the most suitable wavelength.

Method Validation:

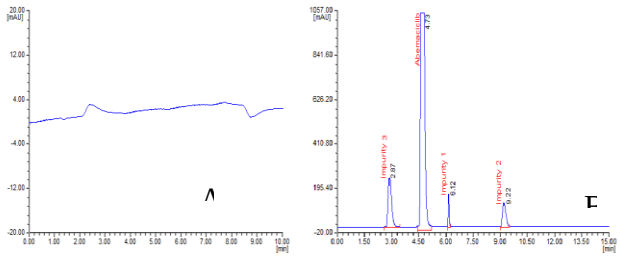
The method developed in the study was validated for its acceptable performance to ensure suitability of indent purpose. The parameters such as range, linearity, accuracy, precision, specificity, detection limit,

quantification limit, ruggedness and robustness experiments were executed ICH guidelines [19].

Results and Discussions:

Impurity profiling was conducted to detect both known and unknown impurities in the pure drug, which is essential for ensuring the safety of pharmaceutical products. A review of the existing literature indicated the absence of an analytical method for quantifying the process-related impurities of abemaciclib. Consequently, this study was undertaken to establish a direct HPLC method for the identification and quantification of process-related impurities 1, 2 and 3 in abemaciclib.

Under these conditions, it was observed that the peaks corresponding to abemaciclib and its impurities exhibited symmetrical shapes, and the resolution between adjacent peaks exceeded 2. Identification of the compounds in the standard solution was achieved by injecting individual standard solutions and comparing their retention times with the standard. The retention times were as follows: 4.73 min for abemaciclib, 6.12 min for impurity 1, 9.22 min for impurity 2, and 2.67 min for impurity 3. Column efficiency assessments for abemaciclib and all its impurities revealed values that were within acceptable limits for theoretical plates and resolution, while exceeding the limit for tail factor. These results are presented in Table 1. Chromatograms of the placebo and the standard abemaciclib solution spiked with impurities are depicted in Figure 3. Based on these chromatograms, it was confirmed that the method was specific for the separation and detection of process-related impurities of abemaciclib.



(a) Place bochromatogram; (b)standard chromatogram

Figure 3: Specificity chromatograms in the proposed method

In every method validation run, the system suitability of the peaks representing abemaciclib and its impurities was confirmed, and the acceptance criteria for each validation run included the following: theoretical plates (N) exceeding 2000, resolution (RS) between adjacent peaks greater than 2, and a tailing factor (AS) of no more than 2.0. The values obtained for N, RS, and AS for the peaks associated with abemaciclib and its impurities fell within the acceptable limits (Table 1), indicating that the optimized method is suitable for the analysis of these compounds.

Table1:System suitability results in the proposed method

Parameter	Results			
	Abemaciclib	Impurity1	Impurity2	Impurity3
System suitability$				
t_R(min)	4.73	6.12	9.22	2.87
RRT	--	1.29	1.95	0.61
R_S	7.46	6.91	12.28	--
A_S	1.03	0.95	0.91	1.07
N	6401	8325	12043	5834

The method's sensitivity for detecting impurities was assessed by determining the Limit of Detection (LOD) and Limit of Quantification (LOQ) of the impurities using the signal-to-noise (s/n) ratio method. The obtained results established LOD as 0.010 µg/mL and LOQ at 0.03 µg/mL for impurities, affirm the method's high sensitivity and method shows capability to detect analytes at extremely low concentrations.

The accuracy of the method was determined based on the recovery (R %) of known amounts of analyte in a placebo sample and was calculated using the following formula

$R\% = C_{found} \times 100 / C_{taken}$

The accuracy of the method was assessed by performing three consecutive replicate injections of control samples at concentrations of 90 µg/mL, 120 µg/mL, and 150 µg/mL for abemaciclib, each spiked with 0.1% of the studied impurities. The acceptable % recovery fell withintherangeof98-102%for both abemaciclib and the studied impurities. Additionally, the % RSD at each spiked concentration level was found to be less than 2%, which is well within the acceptable limit, thus confirming the accuracy of the method. A summary of the results obtained from system suitability, linearity, precision, accuracy, and sensitivity studies in the proposed method is presented in Table 2.

4. LCMS/MS Characterization of DPs

The characterizing degradation products is a crucial step in pharmaceutical analysis, as it helps identify and understand the stability and potential safety concerns of a drug substance. LCMS/MS is a powerful analytical technique for characterizing degradation products due to its structural elucidation capabilities, sensitivity, specificity, and ability to provide quantitative data. It is a valuable tool in pharmaceutical development, quality control, and research, as well as in environmental and chemical analysis. Hence, the DPs generated during forced degradation of abemaciclib were characterized through LCMS/MS analysis. By observing the retention times of DPs in the forced degradation chromatogram, it was ascertained that six distinct DPs were formed and

were designated as DP 1 to 6 by following the elution sequence observed in the chromatogram.

The DP 1 was generated through based as well as UV light degradation study and was identified at 0.95 min. The molecular ion peak for DP I was detected at an m/z value of 193 (m+1) confirm the molecular weight of DP 1 as 192 g/mol. The initial fragment was generated at an m/z of 108 (m+1) by lose of NH2 group and then the second abundant fragment was noticed at an m/z of 108 due to lose of C4H9N2 from the parent ion. A third fragment with m/z 153 was produced by the loss of C2H2N and another fragment was noticed at m/z of 112 with a total loss of C4H5N2 from the parent ion. Based on the date, the DP 1 was identified as 5-(piperazin-1-ylmethyl) pyridin-2-amine. The proposed fragmentation pathway is illustrated in the figure 6 and mass fragmentation spectra were presented in figure.

Table2: Linearity, precision and accuracy results in the proposed method

Parameter	**Results**			
	Abemaciclib	**Impurity1**	**Impurity2**	**Impurity3**
Linearity				
Rangein µg/mL	30-210	0.03-0.21	0.03-0.21	0.03-0.21
Slope	8691.5	346699	276680	441013
Intercept	1921.6	-168.2	211.67	1890.5
r^2	0.9991	0.999	0.9992	0.9992
Precision(n=6)				
Intraday	0.28	0.19	0.30	1.17
Interday(day1)	1.09	0.30	0.46	1.32
Interday(day2)	0.21	0.40	0.32	0.76
Accuracyat 50%level(n=3)				
Amountadded(µg/mL)	90	0.090	0.090	0.090
Recovered(µg/mL)	88.557	0.089	0.089	0.089
%Recovery	98.40	99.00	98.65	98.71
%RSD	0.38	0.41	0.36	0.65
Accuracyat 100%level(n=3)				
Amountadded(µg/mL)	120	0.120	0.120	0.120
Recovered(µg/mL)	118.608	0.118	0.119	0.120
%Recovery	98.84	98.69	99.31	100.18
%RSD	0.56	0.20	0.56	0.72
Accuracyat150%level(n=3)				
Amountadded(µg/mL)	150	0.150	0.150	0.150
Recovered(µg/mL)	148.690	0.150	0.150	0.150
%Recovery	99.13	99.91	100.12	99.79
%RSD	0.71	1.15	0.66	1.00

5. Conclusion

In conclusion, this study introduced an innovative analytical approach utilizing liquid chromatography (LC) and liquid chromatography-mass spectrometry (LCMS/MS) for the effective resolution, identification, and characterization of minute quantities of degradation products (DPs) of abemaciclib. The method employed Zorbax XDB C18 column and demonstrated successful separation of process-related impurities, including abemaciclib, under specified conditions. The mobile phase composition, consisting of phosphate buffer and methanol or acetonitrile, facilitated efficient separation, and the method exhibited a sensitive detection limit and a linear calibration curve as per the International Conference on Harmonization (ICH) guidelines.

Stress studies conducted on pure abemaciclib revealed its instability under acidic, alkaline, and UV light conditions while maintaining stability in thermal and peroxide environments. Six degradation products (DPs) were identified and characterized through LCMS/MS,

providing insights into their fragmentation patterns and masses. The identified DPs were associated with specific chemical structures, enhancing the understanding of abemaciclib degradation pathways. The proposed approach proved effective for routine analysis, showcasing its utility for both pure drug samples and formulations. It not only facilitated the identification of known impurities but also uncovered previously unknown impurities associated with abemaciclib. The study contributes valuable information for quality control and safety assessments of abemaciclib, essential in the pharmaceutical industry. Overall, this research establishes a robust analytical method for the comprehensive analysis of abemaciclib and its related impurities.

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